

R1 = H or one of the following:

$\beta$ -O Linked to the 1, 2, 3, 4, or 6 position of the adjacent monosaccharide or a linear or branched polysaccharide.

R2 = H or one of the following:

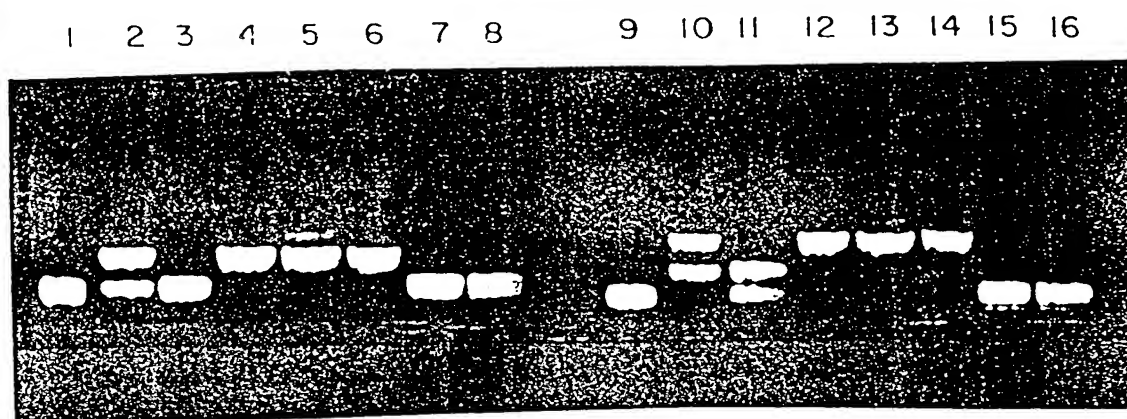
$\alpha$ -O Linked to the 1, 2, 3, 4, or 6 position of the adjacent monosaccharide or a Linear or branched polysaccharide.

R2-10 = H or one of the following: OH, SO<sub>3</sub>, phosphate, NH<sub>2</sub>NHAc, OCH<sub>3</sub>, O-alkyl, CH<sub>3</sub>, CH-alkyl, or inorganic-alkyl; O linked to another R2-10 within the same monosaccharide.

FIG. 1

113 : Gal $\beta$ 1-3G1cNAc $\beta$ 1-3Ga1 $\beta$ 1-4G1c-Co  
/
   
Fuca1-4

167 : Gal $\beta$ 1-3G1cNAc $\beta$ 1-3Ga1 $\beta$ 1-4G1c-Co



#### Substrate 113

- |   |   |                                   |
|---|---|-----------------------------------|
| 1 | + | no preparation                    |
| 2 | + | <u>X. holcicola</u> preparation   |
| 3 | + | <u>X. badrii</u> preparation      |
| 4 | + | <u>X. manihotis</u> preparation   |
| 5 | + | <u>X. cyanopsidis</u> preparation |
| 6 | + | <u>X. oryzae</u> preparation      |
| 7 | + | <u>X. campestris</u> preparation  |
| 8 | + | <u>X. campestris</u> preparation  |

#### Substrate 167

- |    |   |                                   |
|----|---|-----------------------------------|
| 9  | + | no preparation                    |
| 10 | + | <u>X. holcicola</u> preparation   |
| 11 | + | <u>X. badrii</u> preparation      |
| 12 | + | <u>X. manihotis</u> preparation   |
| 13 | + | <u>X. cyanopsidis</u> preparation |
| 14 | + | <u>X. oryzae</u> preparation      |
| 15 | + | <u>X. campestris</u> preparation  |
| 16 | + | <u>X. campestris</u> preparation  |

FIG. 2

109 :  $\downarrow$   
 $\text{Ga1}\alpha 1-3\text{Ga1}\beta 1-3\text{G1cNAc-Co}$

M 1 2 3 4 5 6 7 8 9 10



Substrate 109

Lanes 1-4

1

= complete digest

2

= 1  $\mu\text{l.}$  of  $\alpha 1-3, 6$  Galactosidase

3

= 0.5  $\mu\text{l.}$

4

= 0.25  $\mu\text{l.}$  : concentration of enzyme-4 units/ $\mu\text{l.}$

= 0.125  $\mu\text{l.}$

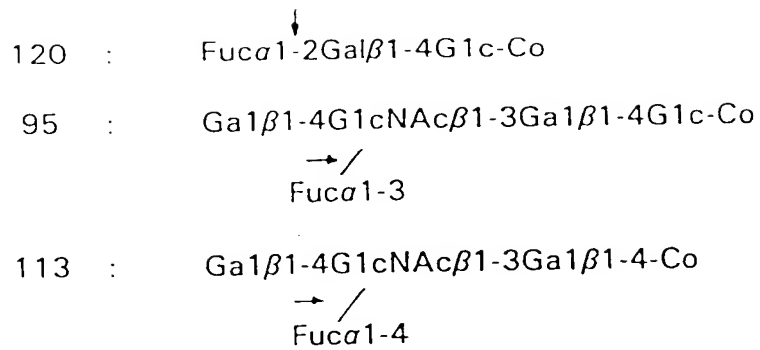
5-8

= partial digest

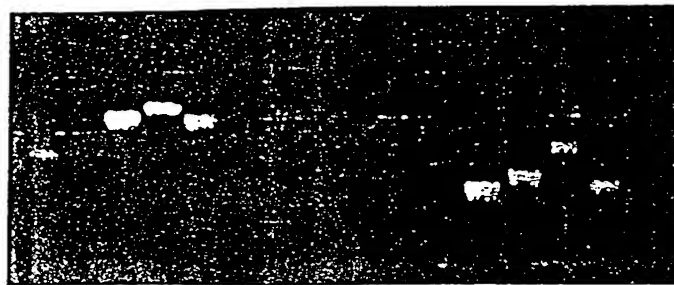
9-10

= undigested

FIG. 3



M 1 2 3 4 5 6 7 8 9 10 11



#### Substrate 120

- 1 + no enzyme
- 2 +  $\alpha$ -Fucosidase II
- 3 +  $\alpha$ -Fucosidase I

#### Substrate 95

- 4 no enzyme
- 5 +  $\alpha$ -Fucosidase I
- 6 +  $\alpha$ -Fucosidase I +  $\beta$ -Galactosidase (bovine testes)
- 7 +  $\alpha$ -Fucosidase II

#### Substrate 113

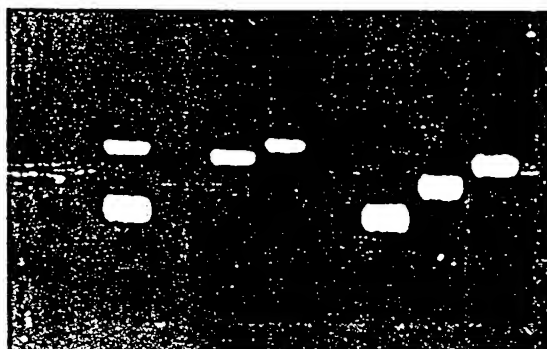
- 8 no enzyme
- 9 +  $\alpha$ -Fucosidase I
- 10 +  $\alpha$ -Fucosidase I +  $\beta$ -Galactosidase (bovine testes)
- 11 +  $\alpha$ -Fucosidase II

FIG. 4

118 : Ga1CnaC $\beta$ 1-4G1cNAc $\beta$ 1-4G1cNAc-Co

167 : Ga1 $\beta$ 1-3G1cNAc $\beta$ 1-3Ga1 $\beta$ 1-4G1c-Co

M 1 2 3 4 5



#### Substrate 118

1 + no enzyme  
2 +  $\beta$ -GlcNAcase

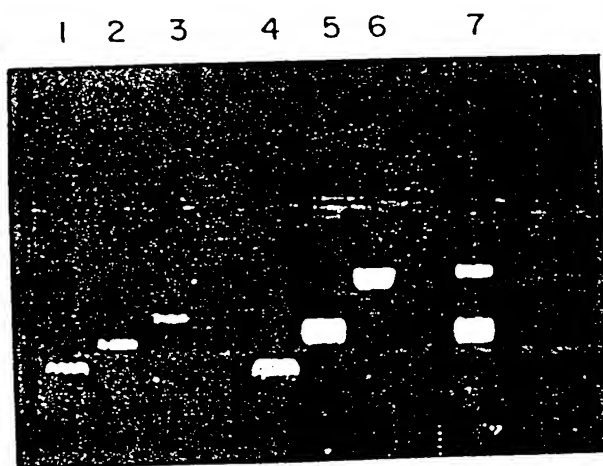
#### Substrate 167

3 + no enzyme  
4 +  $\beta$ -Galactosidase  
5 +  $\beta$ -Galactosidase +  $\beta$ -G1cNAcase

FIG.5

200 :       $\text{Ga1}\beta\text{1-4G1cNAc}\beta\text{1-2Man}\alpha\text{1-6Man}\beta\text{1-4G1c-Co}$   
 (linear)

197 :       $\text{Ga1}\beta\text{1-4G1cNAc}\beta\text{1-6 Ga1}\beta\text{1-4G1c-Co}$   
 (branched)  $\text{Ga1}\beta\text{1-4G1cNAc}\beta\text{1-3}$



## Substrate 200

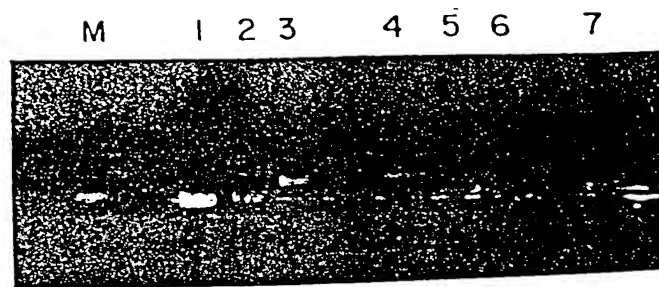
1	+	no enzyme
2	+	$\beta$ -Galactosidase
3	+	$\beta$ -Galactosidase + $\beta$ -GlcNAcase ( <u>X. manihotis</u> )

## Substrate 197

4	+	no enzyme
5	+	$\beta$ -Galactosidase
6	+	$\beta$ -Galactosidase + $\beta$ -G1cNAcase ( <u>X. manihotis</u> )
7	+	Marker (92b,167)

FIG. 6

$\downarrow$   
 96 : Ga1NAc $\beta$ 1-3Ga1 $\alpha$ 1-4Ga1 $\beta$ 1-4G1c-Co  
 $\downarrow$   
 205 : Ga1NAc $\beta$ 1-4Ga1 $\beta$ 1-4G1c-Co



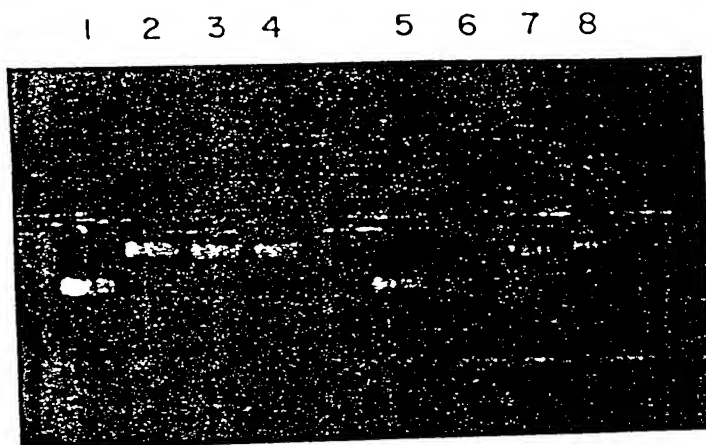
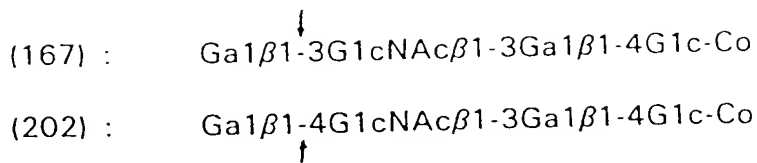
## Substrate 96

1	+	no enzyme
2	+	$\beta$ -GlcNAcase ( <u>X. manihotis</u> )
3	+	$\beta$ -GlcNAcase (bovine kidney)

## Substrate 205

4	+	no enzyme
5	+	$\beta$ -GlcNAcase ( <u>X. manihotis</u> )
6	+	$\beta$ -GlcNAcase (bovine kidney)
7	+	Marker (92b, 167)

FIG. 7



## Substrate 167

- |   |   |   |
|---|---|---|
| 1 | + | no enzyme   |
| 2 | + | $\beta$ 1-3 > > 4 Galactosidase ( <u>X. manihotis</u> ) at 1x concentration |
| 3 | + | $\beta$ 1-3, 4 > 6 Galactosidase (bovine testes) at 1x concentration        |
| 4 | + | $\beta$ 1-3, 4 Galactosidase (chicken liver) at 1x concentration            |

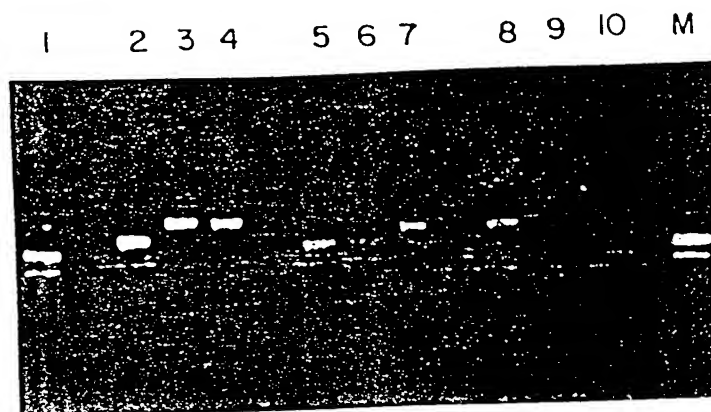
## Substrate 202

- 5 + no enzyme  
6 +  $\beta$ 1-3 > > 4 Galactosidase (X. manihotis) at 100x concentration  
7 +  $\beta$ 1-3, 4 > 6 Galactosidase (bovine testes) at 1x concentration  
8 +  $\beta$ 1-3, 4 Galactosidase (chicken liver) at 1x concentration

FIG. 8



109 :       $\text{Ga1}\alpha\text{1-3Ga1}\beta\text{1-3G1cNAc-Co}$   
 193 :       $\text{Ga1}\alpha\text{1-4Ga1}\beta\text{1-4Gal-Co}$   
 181 :       $\text{Ga1}\alpha\text{1-6G1c}\alpha\text{1-2Fru-Co}$



1              Marker

Substrate 109

2      +      no enzyme  
 3      +       $\alpha\text{1-3, 6 Galactosidase (X. manihotis)}$   
 4      +       $\alpha\text{1-3, 4, 6 Galactosidase (coffee bean)}$

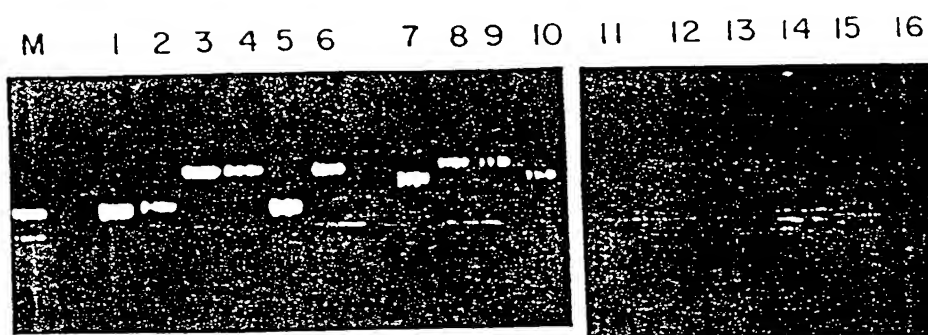
Substrate 193

5      +      no enzyme  
 6      +       $\alpha\text{1-3, 6 Galactosidase (X. manihotis)}$   
 7      +       $\alpha\text{1-3, 4, 6 Galactosidase (coffee bean)}$

Substrate 181

8      +      no enzyme  
 9      +       $\alpha\text{1-3, 6 Galactosidase (X. manihotis)}$   
 10     +       $\alpha\text{1-3, 4, 6 Galactosidase (coffee bean)}$

FIG. 9

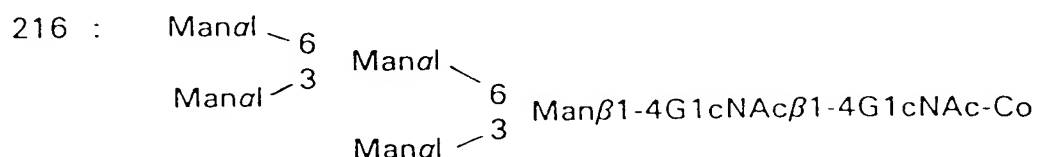
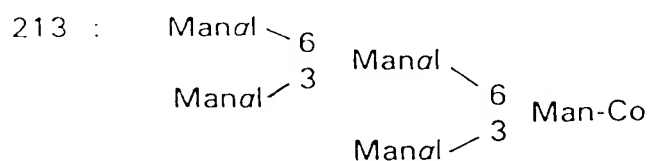


1	+	no enzyme
2	+	$\alpha$ -Mannosidase I (15 units, 20 hrs.)
3	+	$\alpha$ -Mannosidase III (15 units, 2 hrs.)
4	+	$\alpha$ -Mannosidase III (15 units, 20 hrs.)
5	+	$\alpha$ -Mannosidase II (100 units, 20 hrs.)
6	+	Jack bean $\alpha$ -Mannosidase

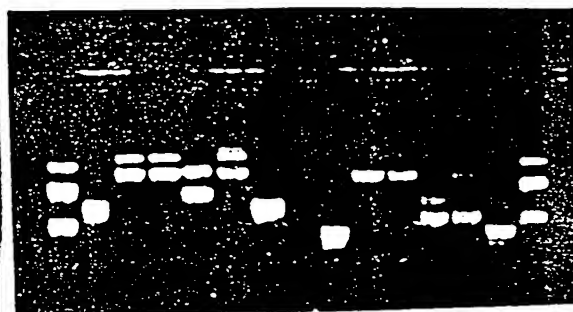
7	+	no enzyme	
8	+	$\alpha$ -Mannosidase I	(15 units, 2 hrs.)
9	+	$\alpha$ -Mannosidase III	(15 units, 2 hrs.)
10	+	$\alpha$ -Mannosidase III	(15 units, 2 hrs.)
11	+	$\alpha$ -Mannosidase II	(100 units, 20 hrs.)

12	+	no enzyme	
13	+	$\beta$ -Galactosidase (bovine testes <sup>OGSI</sup> )	
14	+	$\beta$ -Galactosidase + $\beta$ -GlcNAcase	
15	+	$\beta$ -Galactosidase + $\beta$ -GlcNAcase + $\alpha$ -Mannosidase I units, 2 hrs.)	(15)
16	+	$\beta$ -Galactosidase + $\beta$ -GlcNAcase + $\alpha$ -Mannosidase III units, 2 hrs.)	(15)
17	+	$\beta$ -Galactosidase + $\beta$ -GlcNAcase + $\alpha$ -Mannosidase II units, 2 hrs.)	(15)

FIG. 10



M 1 2 3 4 5 6     7 8 9 10 11 12 M     M 13 14 15 16 17 18     19 20 <sup>21</sup> <sup>23</sup> 22 <sup>24</sup>



## TWO-HOUR INCUBATION

## Substrate 213

- 1 + no enzyme
- 2 +  $\alpha$ -Mannosidase I
- 3 +  $\alpha$ -Mannosidase I + II
- 4 +  $\alpha$ -Mannosidase III
- 5 +  $\alpha$ -Mannosidase II + III
- 6 +  $\alpha$ -Mannosidase II

## Substrate 216

- 7 + no enzyme
- 8 +  $\alpha$ -Mannosidase I
- 9 +  $\alpha$ -Mannosidase I + II
- 10 +  $\alpha$ -Mannosidase III
- 11 +  $\alpha$ -Mannosidase II + III
- 12 +  $\alpha$ -Mannosidase II

## TWENTY-HOUR INCUBATION

## Substrate 213

- 13 + no enzyme
- 14 +  $\alpha$ -Mannosidase I
- 15 +  $\alpha$ -Mannosidase I + II
- 16 +  $\alpha$ -Mannosidase III
- 17 +  $\alpha$ -Mannosidase II + III
- 18 +  $\alpha$ -Mannosidase II

## Substrate 216

- 19 + no enzyme
- 20 +  $\alpha$ -Mannosidase I
- 21 +  $\alpha$ -Mannosidase I + II
- 22 +  $\alpha$ -Mannosidase III
- 23 +  $\alpha$ -Mannosidase II + III
- 24 +  $\alpha$ -Mannosidase II

FIG. 11

12/17

179 : Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-Co

180 : Glc $\alpha$ 1-4Glc $\alpha$ 1-4Glc-Co

118 : GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-Co

202 : Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-Co



M Marker

Substrate 179

1 + no enzyme

2 +  $\beta$ Glucosidase (1 unit)

Substrate 180

3 + no enzyme

4 +  $\beta$ Glucosidase (5 units)

Substrate 118

5 + no enzyme

6 +  $\beta$ Glucosidase (5 unit)

7 +  $\beta$ glcNAcase

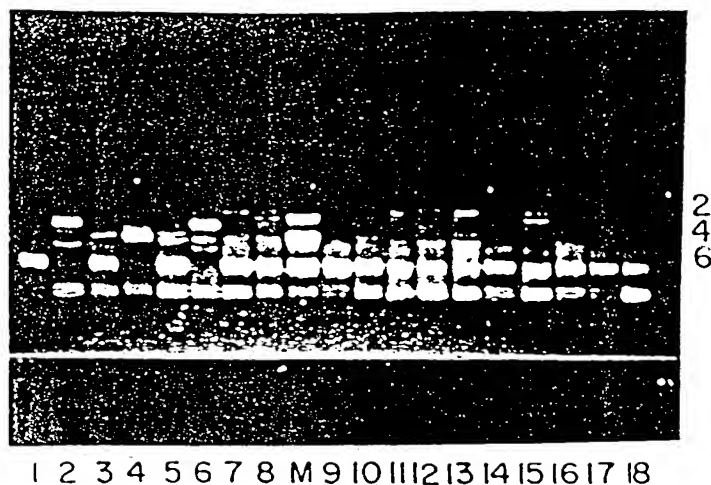
Substrate 202

8 + no enzyme

9 +  $\beta$ Glucosidase (5 units)

10 +  $\beta$ Galactosidase

FIG. 12



Substrate: Gs 300

Lane Nos.

1. No extract
2. *Xanthomonas holycicola* ATCC # 13461
3. *Xanthomonas badrii* ATCC # 11672
4. *Xanthomonas manihotis* ATCC # 49764
5. *Xanthomonas cyanopsidis* ATCC # 55472
6. *Xanthomonas oryzae* ATCC # 55470
7. *Xanthomonas campestris* ATCC # 55470
8. *Xanthomonas campestris*

M: Markers (92b, 167, 197)

9. No extract
10. *Bacillus globigii* I
11. *Bacillus globigii* II
12. *Bacillus caldolyticus*
13. *Bacillus brevis*
14. *Bacillus stearothermophilus* Strain A
15. *Bacillus stearothermophilus* Strain B
16. *Bacillus aneurinolyticus*
17. *Bacillus sphaericus*
18. *Bacillus stearothermophilus* Strain C

FIG. 13

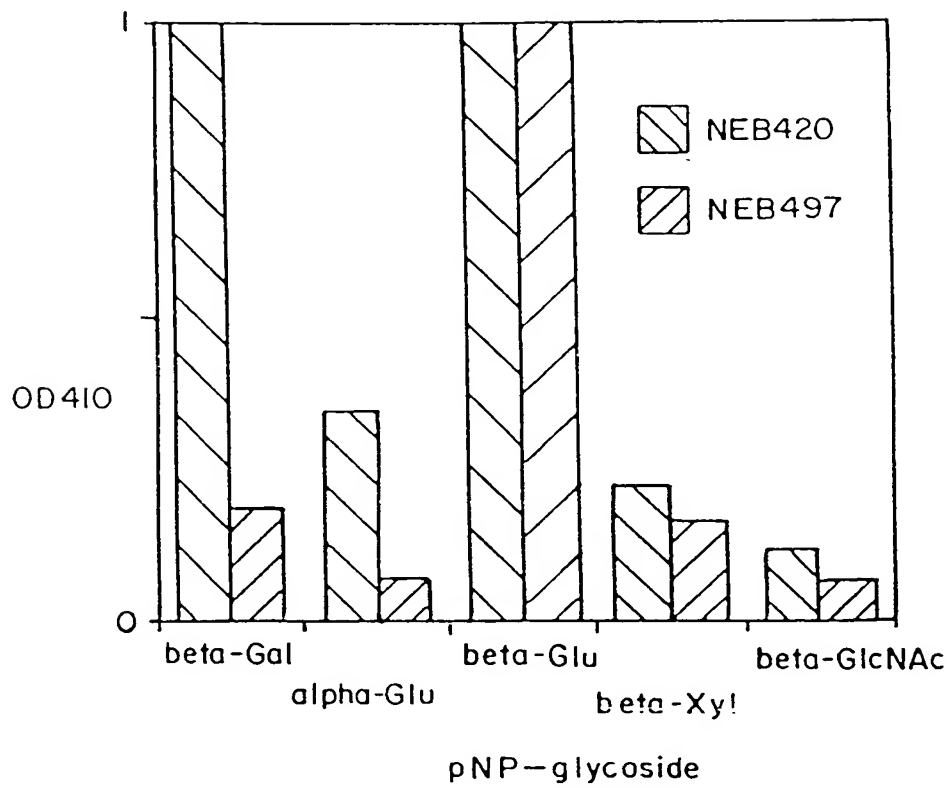
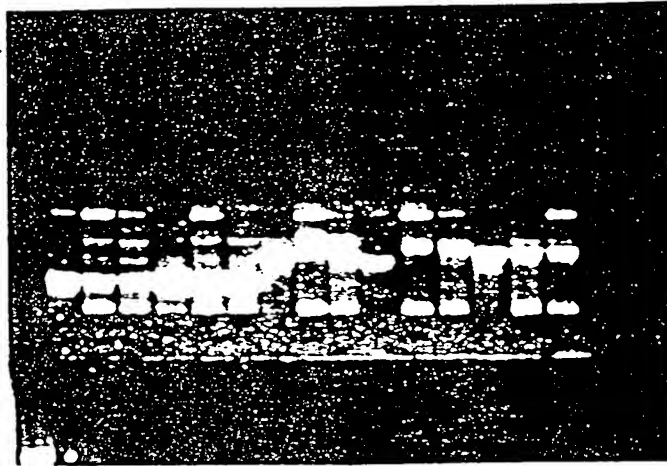


FIG. 14



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

## Substrate 202

- |                                  |         |
|----------------------------------|---------|
| 1. No extract                    |         |
| 2. <i>Xanthomonas campestris</i> | NEB 420 |
| 3. <i>Xanthomonas campestris</i> | NEB 497 |

## Substrate 167

- |                                  |         |
|----------------------------------|---------|
| 4. No extract                    |         |
| 5. <i>Xanthomonas campestris</i> | NEB 420 |
| 6. <i>Xanthomonas campestris</i> | NEB 497 |

## Substrate 180

- |                                  |         |
|----------------------------------|---------|
| 7. No extract                    |         |
| 8. <i>Xanthomonas campestris</i> | NEB 420 |
| 9. <i>Xanthomonas campestris</i> | NEB 497 |

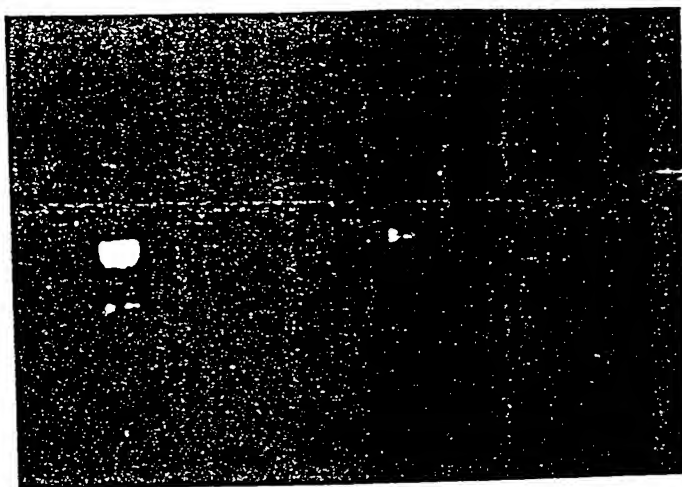
## Substrate 179

- |                                   |         |
|-----------------------------------|---------|
| 10. No extract                    |         |
| 11. <i>Xanthomonas campestris</i> | NEB 420 |
| 12. <i>Xanthomonas campestris</i> | NEB 497 |

## Substrate 233

- |                                   |         |
|-----------------------------------|---------|
| 13. No extract                    |         |
| 14. <i>Xanthomonas campestris</i> | NEB 420 |
| 15. <i>Xanthomonas campestris</i> | NEB 497 |

FIG. 15



M: Marker (191, 202)

Substrate 300

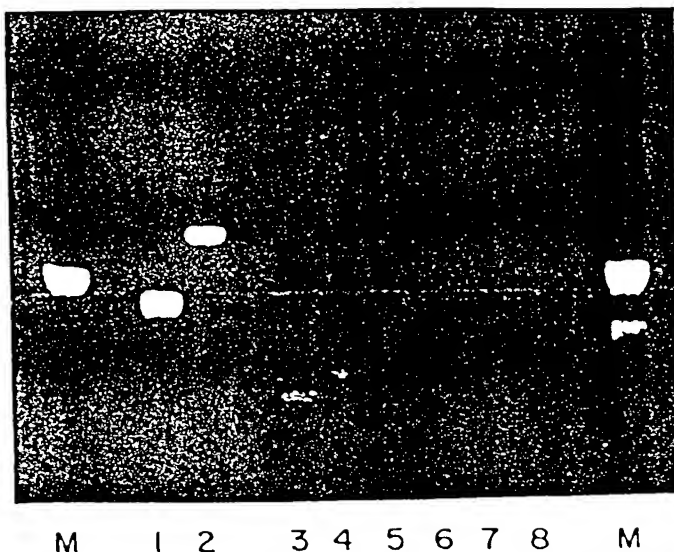
1. No enzyme
2. 2 units  $\alpha$ 1-2, 3 Mannosidase (*Xanthomonas manihotis*)
3. 2 units  $\alpha$ 1-2, 3 Mannosidase + 5 units  $\beta$ -Xylosidase (*Xanthomonas holcicola*)
4. 5 units  $\beta$ -Xylosidase (*Xanthomonas holcicola*)

Substrate 264

1. No enzyme
2. 5 units  $\beta$ -Xylosidase (*Xanthomonas holcicola*)

FIG. 16





M: Marker (191, 202)

Substrate 259

1. No enzyme
2. 2.5 units  $\beta$ -Mannosidase

Substrate 300

3. No enzyme
4. 2 units  $\alpha$ 1-2, 3 Mannosidase (*Xanthomonas manihotis*)
5. 2 units  $\alpha$ 1-2, 3 Mannosidase + 2 units  $\beta$ -Xylosidase (*Xanthomonas holcicola*)
6. 2 units  $\alpha$ 1-2, 3 Mannosidase + 2 units  $\beta$ -Xylosidase + 10 units  $\alpha$ 1-6 Mannosidase (*Xanthomonas manihotis*)
7. 2 units  $\alpha$ 1-2, 3 Mannosidase + 2 units  $\beta$ -Xylosidase + 10 units  $\alpha$ 1-6 Mannosidase + 2.5 units  $\beta$ -Mannosidase (*Xanthomonas holcicola*)
8. 2.5 units  $\beta$ -Mannosidase (*Xanthomonas holcicola*)

FIG. 17